MicroPET Imaging of Bacterial Infection with Specifically Nitroreductase Responsive ^{18}F -labeled Nitrogen Mustard Analogues

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Abstract

Purpose

Bacterial infection and antibiotic-resistant are still serious threats to human health. Here, we aim to develop two novel radiotracers ^{18}F -NTRP and ^{18}F -NCRP with specific nitroreductase (NTR) responsive to image the deep-seated bacterial infection with PET for differentiating infection from sterile inflammation.

Methods

^{18}F -NTRP and ^{18}F -NCRP were synthesized via a one-step method. All the steps of tracers radiosynthesis were successfully adapted in the All-In-One automated module. After the physiochemical properties of ^{18}F -NTRP and ^{18}F -NCRP were characterized, their specificity and selectivity to NTR were verified in *E. coli* and *S. aureus* as well. The *ex vivo* biodistribution of tracers was evaluated in normal mice. MicroPET-CT imaging was acquired in mouse models of bacterial infection and inflammation after the administration of ^{18}F -NTRP and ^{18}F -NCRP.

Results

Fully automated radiosynthesis of ^{18}F -NTRP and ^{18}F -NCRP were achieved within 90 - 110 min with overall decay-uncorrected isolated radiochemical yields of $21.24 \pm 4.25 \%$ and $11.3 \pm 3.78 \%$ respectively. Molar activities of ^{18}F -NTRP and ^{18}F -NCRP were $320 \pm 40 \text{ GBq}/\mu\text{mol}$ and $275 \pm 33 \text{ GBq}/\mu\text{mol}$ respectively. In addition, ^{18}F -NTRP and ^{18}F -NCRP exhibited good selectivity and specificity to NTR response. PET-CT imaging in bacterial infected mice models with ^{18}F -NTRP and ^{18}F -NCRP showed significant radioactivity uptakes in both *E. coli* and *S. aureus* infected muscles. Especially, the *E. coli* infected muscle uptakes were up to $2.4 \pm 0.2 \text{ \%ID/g}$ (^{18}F -NTRP) and $4.05 \pm 0.49 \text{ \%ID/g}$ (^{18}F -NCRP), 3-fold to control uptake. Furthermore, both ^{18}F -NTRP and ^{18}F -NCRP showed a 2.6-fold higher uptake in bacterial infection compared to inflammation counterparts, indicating that they can distinguish infection from inflammation effectively.

Conclusion

Based on the promising results of this preliminary study, ^{18}F -NTRP and ^{18}F -NCRP are worth further study to verify their potentials for bacterial infection imaging via NTR responsive.

Introduction

Although numerous antibiotics have been developed and used in the treatment of bacterial infections for near a century, bacterial infections and multidrug-resistant bacteria are still pressing public health

concerns[1, 2]. Increasing numbers of multidrug-resistant bacteria may be due to the overuse of antibiotics and increased potential risks from invasive surgery or therapy processes[3]. For decreasing the unnecessary usage of antibiotic therapy, a critical issue is differentiating bacterial infection from sterile inflammation accurately, because the treatments for infection and sterile inflammation are diametrically different in the clinic. However, rapid and accurate technologies for early detection and localization of infections in the patient remain a challenge. Traditional tools such as microscopy based on culturing of tissue biopsies or blood samples are not accurate enough, because of the sampling error/bias, furthermore, such methods are most suitable for the later stage of infection[4]. Therefore, noninvasive imaging methods are necessary, and structural imaging techniques such as X-ray, computed tomography (CT), magnetic resonance imaging (MRI) have been applied in infection diagnosis. These techniques are based on the presence of abnormal fluid that occurred late within tissues. However, the abnormal fluid may also appear under but sterile inflammatory conditions[5]. Therefore, these techniques are nonspecific, cannot differentiate infections from inflammation[6].

Nuclear medicine imaging, positron emission tomography (PET), and single positron emission tomography (SPECT) have also been used in infection diagnosis currently[7]. Combined with CT or MRI for anatomical reference, PET-CT, PET-MRI, or SPECT-CT can provide unprecedented sensitivity (nano-to picomolar) and quantifiable readout to deep-seated infections[8–10]. Moreover, PET/SPECT imaging is excelling at visualizing infectious pathophysiology in the early stage beyond structural imaging and evaluating the efficacy of therapeutic regimens. Consequently, many bacteria-specific radiotracers designed for infection imaging have been developed. Several reviews have summarized the metabolic pathways and molecule targets in bacteria of the available radiotracers[11–13]. In general, radiotracers designed for infection imaging are building upon the following aspects in bacteria: (1) Carbohydrate catabolism: 2-¹⁸F-fluorodeoxysorbitol (¹⁸F-FDS)[14, 15], ¹⁸F-maltohexaose (MH¹⁸F)[16], and 6"-¹⁸F-fluoromaltotriose[17]; (2) Bacteria folate biosynthesis: ¹¹C-*para*-aminobenzoic acid (¹¹C-PABA)[18], 2-¹⁸F-*para*-aminobenzoic acid (2-¹⁸F-PABA)[5, 19], and ¹⁸F-Fluoropropyl-trimethoprim (¹⁸F-FPTMP)[20]; (3) Iron transport systems in bacteria: ⁶⁸Ga-pyoverdine PAO1(⁶⁸Ga-PVD-PAO1)[21]; (4) Bacterial thymidine kinase in bacteria: ^{124/125}I-1-(2'-Deoxy-2'-fluoro-β-D-arabinofuranosyl)-5-iodoracil (^{124/125}I-FIAU)[22]; (5) Components of cell wall: D-methyl-¹¹C-methionine (¹¹C-D-Met)[22], D-3-¹¹C-Alanine[23]; (6) Antimicrobial peptides: ^{99m}Tc-UBI-29-41[24] and ⁶⁸Ga-UBI-29-41[25]. Although many bacteria-specific tracers have been developed, some of them exhibit good outcomes in infection imaging and show strong clinic potential. However, there are also limitations of these tracers, such as low specificity in patients, limited differentiating ability of particular bacterial strains and dependent on bacterial metabolic state for some tracers. Thus, it is important to exploit potential targets of bacteria for infection imaging.

In this study, bacterial nitroreductase (NTR) was selected as a new target for infection imaging. NTR is a family of flavin-containing enzymes, widely exist in most Gram-positive, Gram-negative bacteria and hypoxia tumors[26, 27]. Aromatic nitro groups can be reduced to nitroso group, hydroxylamine group, and the amino group ultimately under the catalysis of NTR[28]. In this reductive reaction, reduced nicotinamide adenine dinucleotide (NADH) or nicotinamide adenine dinucleotide phosphate (NADPH) is a

vital cofactor, provide electrons in the reduction process[29]. Notably, NTR is also recognized as a biomarker of bacterial infection in clinical, food, and environmental samples. Up to now, NTR-activated imaging tools have attracted great attention for infection imaging, include electrochemical and fluorescent imaging[30–32]. Although NTR activated optical imaging probes used in infection imaging have already been well described and studied, radiotracers responsive to bacterial NTR for detecting and localizing deep-seated infections *in vivo* are still scarce.

Herein, we designed and radiolabeled two NTR activated tracers, nitrogen mustard analogues decorated with aromatic nitro groups (^{18}F -NTRP and ^{18}F -NCRP), as new PET tracers applied for infection imaging. ^{18}F -NTRP and ^{18}F -NCRP are consists of two separate functional domains: an efficient NTR responsive moiety “aromatic nitro group” and a potential trapped effector “nitrogen mustard”. Nitro is a kind of strong electro-withdrawing group, decreases the electron density of nitrogen mustard required for alkylation. In bacterial infection sites, the nitro group of ^{18}F -NTRP or ^{18}F -NCRP is likely to be reduced to electron-donating group amino with NTR specifically. After that, the lone pair electrons developed on nitrogen mustard are conducive to the formation of a highly electrophilic aziridinium ring, which enables cross-link to bacterial DNA through covalent bond.

In this study, we developed fully automated radiosynthesis methods of ^{18}F -NTRP and ^{18}F -NCRP, which consistently yielded the final products in large quantity with high radiochemical purity (RCP) and molar activity. Both ^{18}F -NTRP and ^{18}F -NCRP were specifically reduced by NTR and readily taken up and retained by bacteria. *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*) were chosen as representative Gram-negative and Gram-positive bacteria and typically overexpress NTR[33]. PET imaging in *E. coli* and *S. aureus* infected mice models were also performed with ^{18}F -NTRP and ^{18}F -NCRP. In addition, many frequently encountered infections are caused by *E. coli* and *S. aureus*, such as pneumonia, diarrhea, superinfection, and osteomyelitis, etc.[34, 35]. We also performed PET imaging noninvasively differentiate infection *in vivo* from sterile inflammation with ^{18}F -NTRP and ^{18}F -NCRP. Therefore, we think ^{18}F -NTRP and ^{18}F -NCRP having outstanding performance in early detecting, and specifically locating infections *in vivo*.

Materials And Methods

Detailed materials and methods for synthesis, characterization, *in vitro* and *in vivo* evaluations of tracers were described in the ESI section.

Results And Discussion

Radiochemical Synthesis of ^{18}F -NTRP and ^{18}F -NCRP

We designed and synthesized two PET tracers, ^{18}F -NTRP and ^{18}F -NCRP, for infection imaging *in vivo*. The overall rationale of ^{18}F -NTRP and ^{18}F -NCRP for infection imaging is that radiotracers enable the response

to bacterial NTR specifically and then be trapped in living bacteria. The structure of tracers is aryl nitrogen mustard decorated with a *para*-NTR-activated nitro aromatic group (Scheme 1). Electron-withdrawing nitro group decreases the electron density of the benzene ring efficiently and makes the lone pair electrons of the nitrogen mustard delocalized to nitro. Thereby, impeding the formation of an electrophilic aziridinium ring and resulting in the nitrogen mustard group being unable to connect to DNA. However, in the infection site, the radiotracer is specifically reduced by bacterial NTR, the nitro transforms into electron-donating amino, which triggers an increase of electron density to a mustard moiety, and promotes the formation of aziridinium ring. The electrophilic aziridinium ring is prone to cross-link to bacterial DNA through a covalent bond, resulting in radioactivity being trapped in bacteria. Herein, we synthesized two PET tracers of ^{18}F -NTRP and ^{18}F -NCRP with different labeling positions and nitrogen mustard groups to investigate the structure-function relationship (Scheme 1).

The synthetic strategy was devised to synthesize the precursors and reference compounds of ^{18}F -NTRP and ^{18}F -NCRP according to the routes depicted in Figures S1 and S2 with high yields. Detailed synthetic procedures and full characterization data of intermediates, precursors, and reference standards can be found in the ESI section. ^{18}F -NTRP was synthesized with a tosylate precursor via a one-step method and ^{18}F -NCRP was radiolabeled with a nitro group precursor at a mild situation following the literature[36]. All the steps of tracer radiosynthesis were achieved in 90 - 110 min, including the initial receiving, eluting, and drying of [^{18}F]fluoride that from cyclotron target water, probes radiolabeling via nucleophilic substitution reaction, product purification, and injections formation were successfully adapted in the All-In-One automated module (Figures S3 and S4). Final clear injections were obtained with a pH of 6 - 7. Starting from 14.8 – 25.9 GBq of [^{18}F]fluoride, the overall decay-uncorrected radiochemical yields (RCY) were $20.24 \pm 6.25 \%$ ($n = 13$) for ^{18}F -NTRP and $11.3 \pm 3.78 \%$ ($n = 12$) for ^{18}F -NCRP respectively. Therefore, robust and fully automated radiosynthesis methods have been developed, which can be readily scaled up for the production of multiple clinical doses. The molar activities of ^{18}F -NTRP and ^{18}F -NCRP were $320 \pm 40 \text{ GBq}/\mu\text{mol}$ ($n = 13$) and $275 \pm 33 \text{ GBq}/\mu\text{mol}$ ($n = 12$) at the end of synthesis (EOS), with high RCP (over 95%). The chemical identities of ^{18}F -NTRP and ^{18}F -NCRP were confirmed by co-injection of the final products with non-radioactive reference standard ^{19}F -NTRP and ^{19}F -NCRP for radio-HPLC analysis. The consistent HPLC retention time of the radioactive tracers and their corresponding non-radioactive reference standards (Figure 1A) suggested that the chemical structures of ^{18}F -NTRP and ^{18}F -NCRP were correct. In addition, both ^{18}F -NTRP and ^{18}F -NCRP showed excellent stability *in vitro* after 2 h incubation in saline or serum (Figures 1B, 1C). Taken together, these newly developed ^{18}F -NTRP and ^{18}F -NCRP tracers were obtained with high RCP, molar activity and excellent stability consistently, which satisfied the requirement of potential clinical applications.

Specificity and Selectivity to NTR

Since ^{18}F -NTRP and ^{18}F -NCRP were designed to a specific response to bacterial NTR, we next investigated the specificity and selectivity of the tracers to NTR. We first studied the specificity of ^{18}F -

NTRP and ^{18}F -NCRP to NTR in a Tris-buffered solution. The amino-substituted species ^{19}F -NTRPH and ^{19}F -NCRPH were synthesized as the reference standard to analyze the reduced productions (Figures S1, S2). After reduction, we found that in the presence of NTR and NADPH, 88 % of ^{18}F -NTRP have been reduced, and 37 % of reduced products were ^{18}F -NTRPH (Figure 2A). Similar results were observed in ^{18}F -NCRP and NTR solution, in which 89 % of ^{18}F -NCRP have been reduced, and 17 % was translated to ^{18}F -NCRPH (Figure 2B). These results demonstrated that ^{18}F -NTRP and ^{18}F -NCRP were specifically responsive to NTR *in vitro*. Even though most of the parent tracers had been reduced, the transformation rate of ^{18}F -NTRP to ^{18}F -NTRPH and ^{18}F -NCRP to ^{18}F -NCRPH were relatively low. We speculate that the low transformation rate may be due to a portion of the reduced products in the solution were oxidized to the corresponding parent compound or intermediates under the atmosphere. Additionally, we also carried out the selective study of ^{18}F -NTRP and ^{18}F -NCRP to various species. We compared the transformation rate of ^{18}F -NTRP to ^{18}F -NTRPH and ^{18}F -NCRP to ^{18}F -NCRPH in different kinds of small molecule solutions. As shown in Figures 2C and 2D, ^{18}F -NTRP and ^{18}F -NCRP displayed an excellent selectivity towards NTR. The transformation rate of ^{18}F -NTRP to ^{18}F -NTRPH and ^{18}F -NCRP to ^{18}F -NCRPH incubated in numerous species was about 0.7 – 1.3 %, similar to that observed in saline. However, in the presence of NTR and NADPH, the transformation rate was 13 – 28-fold higher than achieved with other species, even though the concentration of NTR was much lower than with other species (0.2 mM vs 50 mM). These results indicated that ^{18}F -NTRP and ^{18}F -NCRP have good specificity to NTR and exhibited excellent anti-interference ability.

Bacterial Uptake Studies

We subsequently evaluated the bacterial uptake and retention of ^{18}F -NTRP and ^{18}F -NCRP in representative human pathogens *E. coli* and *S. aureus* *in vitro* to determine whether tracers could be internalized and trapped in bacteria. As shown in Figures 3A and 3B, obviously much higher uptakes of ^{18}F -NTRP and ^{18}F -NCRP with both living Gram-negative (*E. coli*) and Gram-positive (*S. aureus*) bacteria were observed than that with heat-killed bacteria. Moreover, ^{18}F -NTRP showed increased accumulation in living bacteria with time, the uptakes reached 3.64 ± 0.46 % in *E. coli* and 2.76 ± 0.23 % in *S. aureus* at 90 min, which were about 5 – 6-fold higher than the controls (0.62 ± 0.11 % and 0.55 ± 0.18 % in heat-killed *E. coli* and *S. aureus*, respectively). In addition, *E. coli* exhibited a 1.3-fold uptake of that achieved by *S. aureus*. Possibly due to the higher levels of NTR expressed in *E. coli* than in *S. aureus*. Compared to ^{18}F -NTRP, ^{18}F -NCRP demonstrated higher incorporation in both living *E. coli* and *S. aureus* (7.71 ± 0.73 % and 6.13 ± 0.18 % respectively), which is about a 2-fold higher than seen with ^{18}F -NTRP. The discrepancy of cellular uptake between ^{18}F -NTRP and ^{18}F -NCRP may be due to the ^{18}F -NCRP being able to be incorporated into DNA more firmly because there are two alkyl chains in ^{18}F -NCRP that can cross-link with bacterial DNA, but only one alkyl chain in ^{18}F -NTRP.

Based on the higher uptake of ^{18}F -NCRP in *E. coli*, we further investigated the retention capacity of ^{18}F -NCRP in *E. coli*. Results in Figure 3C showed that ^{18}F -NCRP exhibited good retention in bacteria. After

incubation for 10, 20, and 30 min, there were $81.43 \pm 21.03 \%$, $70.23 \pm 12.63 \%$, and $66.41 \pm 16.96 \%$ radioactivity were retained in *E. coli*, respectively.

PET Imaging

A good cellular uptake and retention of ^{18}F -NTRP and ^{18}F -NCRP provide the basis of PET-CT imaging in deep-seated infections *in vivo*. Thus, we next performed PET-CT imaging of mouse thigh bacterial infection models with ^{18}F -NTRP and ^{18}F -NCRP. At 24 h post-infection with *S. aureus* and *E. coli*, infected mice were injected with 7.4 - 8.14 MBq of ^{18}F -NTRP or ^{18}F -NCRP via tail vein. At 40 min post-injection (p.i.), a single static 30 min PET acquisition was performed. Figure 4A demonstrated that radioactivity derived from ^{18}F -NTRP was well accumulated in both *S. aureus* and *E. coli* infected muscles. Significantly, the uptake was $2.4 \pm 0.2 \%$ ID/g in *E. coli* infected muscles, which was 1.47-fold to that of *S. aureus* infected muscles ($1.63 \pm 0.15 \%$ ID/g) and 4-fold to the uninfected control muscles ($0.65 \pm 0.09 \%$ ID/g) (Figure 4B). The good target-to-control contrast enabled ^{18}F -NTRP to observe infections in deep sites. In addition, the intravital radioactivity uptake by bacteria in infected mice was well consistent with *in vitro* uptake by bacterial culture. We further studied the biodistribution of the radioactivity in infected triceps, both bilateral triceps of mice were excised after PET imaging and followed by autoradiography. Figure S5A showed that obviously infection tissues were observed in bilateral triceps. Moreover, the radioactivity in triceps was mostly accumulated in infection areas and radioactivity in *E. coli* infected tissues was higher than in *S. aureus* infected samples (Figure S5B). In the ^{18}F -NCRP group, PET imaging (Figure 4A) showed that visible accumulation of radioactivity was observed in *E. coli* and *S. aureus* infected muscles. Moreover, low radioactivity uptake was observed in blood, liver, lung, heart, and brain except for the gall bladder, making ^{18}F -NCRP more favorable for a localized infection diagnosis. PET images of ^{18}F -NCRP demonstrated that the radioactivity in *E. coli* infection was $4.05 \pm 0.49 \%$ ID/g, 1.48-fold to that observed in *S. aureus* infection ($2.7 \pm 0.3 \%$ ID/g), and 3.2-fold to uninfected control muscles ($1.24 \pm 0.26 \%$ ID/g). Compared to ^{18}F -NTRP, ^{18}F -NCRP exhibited better properties in bacterial imaging. The uptakes of ^{18}F -NCRP in *E. coli* and *S. aureus* infected tissues were 1.67 and 1.65 times higher than that of ^{18}F -NTRP, respectively (Figure 4B). Significantly, the fast clearance of ^{18}F -NCRP from healthy organs or tissues, which helps generate a high infection-to-background ratio and provides benefits for clinic infection diagnosis. In addition, we found that *E. coli* infected triceps was much more severe than *S. aureus* infected ones, and autoradiography results showed that the radioactivity in *E. coli* infected muscle was also higher than that in *S. aureus* infected obviously (Figure S5C, S5D). Thus, ^{18}F -NCRP imaging could distinguish the severity of infection *in vivo*.

The ability to differentiate active infection from sterile inflammation is a critical requirement for bacteria-specific radiotracers. Hence, we developed a mixed infection and inflammation mice model for PET imaging with ^{18}F -NTRP and ^{18}F -NCRP. We first evaluated the infection and inflammation model with 2-[^{18}F]-Fluoro-2-deoxy-D-glucose (^{18}F -FDG), which is commonly used in clinical diagnosis. PET imaging of ^{18}F -FDG showed that the uptakes in inflamed and infected muscle were $15.47 \pm 2.46 \%$ ID/g and $6.70 \pm$

0.34 %ID/g, respectively (Figure S6). Even though the uptakes of infected and inflamed areas were much higher than normal muscle (2.78 ± 0.77 %ID/g), there is no sufficient discrepancy between infected and inflamed muscles, indicating that ^{18}F -FDG could not differentiate bacterial infection from sterile inflammation effectively *in vivo*. PET imaging with ^{18}F -NTRP or ^{18}F -NCRP was also performed in the same model mice. At 40 min p.i., apparent ^{18}F -NTRP radioactivity was accumulated in *E. coli* infected muscle (1.90 ± 0.30 %ID/g), which is 3-fold to that accumulated in inflamed muscle and 3.8-fold to that seen in healthy control muscle. However, the uptakes between inflamed muscle and healthy control muscle were no significant difference (0.67 ± 0.08 %ID/g vs 0.48 ± 0.07 %ID/g). In the ^{18}F -NCRP group, the uptake ratio of *E. coli* infected muscles to inflammation muscles was 2.5 (3.90 ± 0.30 %ID/g vs 1.52 ± 0.17 %ID/g). Moreover, *E. coli* infection uptake was 3.8-fold to healthy muscle uptake (3.90 ± 0.30 %ID/g vs 1.02 ± 0.22 %ID/g, Figures 4C, 4D). The low uptake in inflammation muscles was in accordance with the cellular uptake results of ^{18}F -NTRP and ^{18}F -NCRP in heated-killed bacteria. That is due to the complete Freund's adjuvant is consisted with heat-killed *M. tuberculosis* to inducing inflammation.

Metabolic Analysis

Next, we analyzed the metabolic stability of ^{18}F -NTRP and ^{18}F -NCRP *in vivo*. Healthy mice were injected with ^{18}F -NTRP or ^{18}F -NCRP (37 – 38 MBq) via tail vein. Blood and urine samples were collected at 2, 10, 30, or 60 min p.i., respectively. Collected samples were preconditioned to remove the biomacromolecules and then were subjected to radio-HPLC analysis. As shown in Figure 5A, for the blood samples, more than 95 % of intact ^{18}F -NTRP was observed with a retention time of 8.24 min at 2 min p.i. At 10 min p.i., about 73.3 % intact ^{18}F -NTRP remained and a small radio-metabolite was detected at 2.43 min on HPLC chromatogram. Moreover, less intact ^{18}F -NTRP was found at 30 min p.i. (40.8 %) and finally no intact ^{18}F -NTRP left at 60 min p.i. At the same time, the radio-metabolite peaked at 2.43 min was increased to 97.51 % from 10 min to 60 min p.i. For the urine samples, no radio-metabolite was detected at the first (2 min p.i.). After that, no intact ^{18}F -NTRP was observed, but a metabolite was observed at 2.69 min with gradually increased concentration from 10 - 60 min p.i. Due to the minor defluorination being observed in the following biodistribution study, we suspect that the radio-metabolite in blood and urine consisted of a little ^{18}F -fluoride and electrophilic aziridinium ring derived from ^{18}F -NTRP. In addition, NTR existing in normal organs promotes the formation of electrophilic aziridinium rings from ^{18}F -NTRP. Compared to ^{18}F -NTRP, ^{18}F -NCRP was cleared from blood more quickly. The percentage of intact ^{18}F -NCRP in the blood sample was decreased to 46.46 % at 2 min p.i., and three metabolites were detected at 3.95 min, 6.82 min, and 9.91 min, respectively. We speculate that these metabolites were three different intermediates of the aromatic nitro group reduced to amino by NTR *in vivo*. From 10 - 30 min p.i., only about 5.63 % intact ^{18}F -NCRP remained in the blood sample and the proportion of radio-metabolite at 3.95 min on HPLC chromatogram was increased to 88.96 %. In the urine sample, an apparent radio-metabolite was detected at 3.67 min on the HPLC chromatogram. In addition, only 0.2 % of parent ^{18}F -NCRP was detected from 2 - 10 min p.i., but it was undetectable after 30 min p.i. (Figure 5B).

Biodistribution Study

Finally, we studied the biodistribution of ^{18}F -NTRP and ^{18}F -NCRP in normal mice. Healthy mice were injected with ^{18}F -NTRP or ^{18}F -NCRP (0.37 MBq/mouse) vial tail vein, followed by sacrificed at desired time points. At 2 min p.i. of ^{18}F -NTRP, high uptakes were observed in NTR expressed tissues, such as liver (15.21 ± 1.20 %ID/g), kidney (16.56 ± 2.22 %ID/g). Most of the rest radioactivity were concentrated in blood (6.02 ± 0.62 %ID/g), heart (7.41 ± 0.64 %ID/g), lung (12.38 ± 1.63 %ID/g), spleen (6.74 ± 0.99 %ID/g), brain (6.80 ± 0.66 %ID/g), and intestines (7.61 ± 0.10 %ID/g). Less radioactivity was accumulated in the stomach (3.36 ± 0.26 %ID/g), bone (2.79 ± 0.41 %ID/g), and muscle (4.88 ± 0.55 %ID/g). At 10 - 60 min p.i., radioactivity was gradually cleared from most organs. Lower muscle uptake is well supporting the muscle infection imaging. However, incorporated radioactivity in the kidney and intestines was increased to 17.33 ± 2.94 %ID/g and 8.61 ± 1.56 %ID/g, which might be due to the main renal and intestines excretion of ^{18}F -NTRP. Notably, we found significant radioactivity in the small and large bowel, especially concentrated in excreta. This background uptake was a result of the rich microorganisms in the enteric canal. Low bone uptakes from 2 min to 60 min p.i. may indicate extremely low defluorination of ^{18}F -NTRP *in vivo* (Figure 6A). For ^{18}F -NCRP, the radioactivity distribution patterns were similar to those observed with ^{18}F -NTRP. At 2 min p.i., high uptakes in blood (7.96 ± 0.32 %ID/g), heart (9.24 ± 0.43 %ID/g), lung (14.00 ± 0.39 %ID/g), spleen (12.65 ± 0.63 %ID/g), brain (8.45 ± 0.17 %ID/g), liver (18.80 ± 0.60 %ID/g), and kidneys (12.72 ± 0.26 %ID/g) were found. Radioactivity derived from ^{18}F -NCRP was cleared quickly from most organs except that kidney uptake was increase to 16.12 ± 0.42 %ID/g at 10 min p.i., followed by decreased to 2.34 ± 0.37 %ID/g at 60 min p.i. Moreover, uptakes in the bone, stomach and intestinal were increased to 11.53 ± 1.22 %ID/g, 9.40 ± 0.23 %ID/g and 5.74 ± 0.49 %ID/g, respectively at 60 min p.i., which is higher than that observed at 2 min p.i. These results indicated that ^{18}F -NCRP had slight defluorination *in vivo* and was mainly excreted by the kidneys (Figure 6B).

Conclusions

In this study, we developed NTR-specific responsive tracers ^{18}F -NTRP and ^{18}F -NCRP for PET imaging of deep-seated bacterial infection. Automated radiosynthesis of tracers were consistently obtained with high RCY, RCP and molar activity. Both tracers displayed high sensitivity NTR response and could be selectively accumulated in living bacteria and bacteria-infected tissue but not in heat-killed organisms. In addition, high contrast PET-CT images of the *in vivo* bacterial infection were obtained with ^{18}F -NTRP and ^{18}F -NCRP, and tracers could effectively distinguish infections from inflammation in the mouse model. The promising results may further enhance our understanding of bacterial infections and NTR-related molecular events *in vivo*.

Declarations

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Conflicts of interest

The authors have no conflicts of interest to declare that are relevant to the content of this article.

Compliance with Ethical Standards

The experimental procedures, animal use, and care protocols were approved by the Institutional Animal Care and Use Committee of Xiamen University.

Author contributions

Conceptualization: Xianzhong Zhang, Lumei Huang, Jianyang Fang, Zhide Guo; Methodology: Roangqiang Zhuang, Lumei Huang, Shouqiang Hong, Xilin Zhao; Formal analysis and investigation: Lumei Huang, Jianyang Fang, Huanhuan Liu, Haotian Zhu, Lixia Feng; Writing - original draft preparation: Lumei Huang, Jianyang Fang; Writing - review and editing: Xianzhong Zhang, Zhide Guo, Xilin Zhao; Funding acquisition: Xianzhong Zhang, Zhide Guo; Supervision: Xianzhong Zhang. All authors read and approved the final manuscript.

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Figures

Figure 1

(A) Radio-HPLC analysis of ^{18}F -NTRP and ^{18}F -NCRP by co-injection with their non-radioactive reference standards; In vitro stabilities of ^{18}F -NTRP and ^{18}F -NCRP after incubated in (B) saline or (C) serum for different times.

Figure 2

HPLC analysis of the reduction of (A) $^{18}\text{F-NTRP}$ to $^{18}\text{F-NTRPH}$ (□: $^{19}\text{F-NTRP}$, □: $^{18}\text{F-NTRP}$, □: $^{19}\text{F-NTRPH}$, □: $^{18}\text{F-NTRP} + \text{NTR} + \text{NADPH}$) and (B) $^{18}\text{F-NCRP}$ to $^{18}\text{F-NCRPH}$ (□: $^{19}\text{F-NCRP}$, □: $^{18}\text{F-NCRP}$, □: $^{19}\text{F-NCRPH}$, □: $^{18}\text{F-NCRP} + \text{NTR} + \text{NADPH}$) with NTR in Tris-buffer; The selectivity analysis of $^{18}\text{F-NTRP}$ (C) and $^{18}\text{F-NCRP}$ (D) to NTR in vitro.

Figure 3

In vitro bacterial uptakes of (A) ^{18}F -NTRP and (B) ^{18}F -NCRP in *E. coli* and *S. aureus* at 15, 30, 60, and 90 min, respectively; (C) Efflux of ^{18}F -NCRP from *E. coli* after incubated for 90 min. H.K. = heat-killed *E. coli* or *S. aureus*.

Figure 4

(A) Representative PET-CT images of infected model mice with ^{18}F -NTRP and ^{18}F -NCRP at 40 min p.i. The *E. coli* (red arrow) and *S. aureus* (white arrow) infected sites were indicated; (B) Uptakes of ^{18}F -NTRP and ^{18}F -NCRP in *E. coli* and *S. aureus* infected tissues were derived from PET-CT images; (C) Representative PET-CT images of mixed infection and inflammation mice model with ^{18}F -NTRP and ^{18}F -NCRP at 40 min p.i. The infected (red arrow) and inflamed triceps (green arrow) were indicated. (D)

Uptakes of ^{18}F -NTRP and ^{18}F -NCRP in the infected and inflamed triceps were derived from PET-CT images.

Figure 5

(A) Metabolic stability of ^{18}F -NTRP in normal mice at 2, 10, 30, and 60 min p.i.; (B) Metabolic stability of ^{18}F -NCRP in normal mice at 2, 10, and 30 min p.i.

Figure 6

Biodistribution of (A) ^{18}F -NTRP and (B) ^{18}F -NCRP in normal mice at 2, 10, 30 and 60 min p.i. Results were expressed as percentage injected activity dose per gram of sample (%ID/g, mean $\pm$ SD).

Supplementary Files

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- [Scheme1.png](#)
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